Atty. Dkt. No. 029318-0799

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: H. William BOSCH et al.

Title: BIOADHESIVE NANOPARTICULATE COMPOSITIONS HAVING CATIONIC SURFACE STABILIZERS

Appl. No.: 10/004,808

Filing Date: 12/07/2001

Examiner: Susan T. Tran

Art Unit: 1615

TRANSMITTAL OF BRIEF ON APPEAL

Mail Stop APPEAL BRIEF-PATENTS
Commissioner for Patents
PO Box 1450
Alexandria, Virginia 22313-1450

Sir:

Transmitted herewith is an appeal brief in the above-identified application:

Submitted herewith in connection with the above application are the following:

[X] Brief on Appeal.

[X] A check in the amount of $500.00 is enclosed in payment of fee for filing a brief in support of an appeal under 37 CFR 1.17(c).

[X] Applicant hereby petitions for an extension of time under 37 C.F.R. §1.136(a) for the total number of months checked below:

[X]	Extension for response filed within the first month:	$120.00		$120.00

[X] A check in the amount of $620.00 is enclosed in payment of fee for filing a brief in support of an appeal under 37 CFR 1.17(c).

[ ] Please charge Deposit Account No. 19-0741 in the amount of $00.00.

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[**X**] The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741. If any extensions of time are needed for timely acceptance of papers submitted herewith, applicant hereby petitions for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extensions fees to Deposit Account No. 19-0741.

Please direct all correspondence to the undersigned attorney or agent at the address indicated below.

Respectfully submitted,

Date August 16, 2005

By _____

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Atty. Dkt. No. 029318-0799

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Applicant: H. William BOSCH et al.

Title: ***BIOADHESIVE NANOPARTICULATE COMPOSITIONS HAVING CATIONIC SURFACE STABILIZERS***

Appl. No.: 10/004,808

Filing Date: 12/7/2001

Examiner: Susan T. Tran

Art Unit: 1615

BRIEF ON APPEAL UNDER 37 C.F.R. § 41.37

Mail Stop Appeal Brief - Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Under the provisions of 37 C.F.R. § 41.37, this Appeal Brief is being filed together with a check in the amount of $500.00 covering the 37 C.F.R. 41.02(b)(2) appeal fee. If this fee is deemed to be insufficient, authorization is hereby given to charge any deficiency (or credit any balance) to the undersigned deposit account 19-0741.

I. REAL PARTY IN INTEREST

The real party in interest is Elan Pharma International, LTD., to whom the inventors assigned their right, title, and interest in this application, by executing an assignment recorded on December 30, 1999, at Reel/Frame 010500/0223 in the United States Patent and Trademark Office. This application is a divisional of Application No. 09/414,159, which was filed on October 8, 1999, now U.S. Patent No. 6,428,814.

II. RELATED APPEALS AND INTERFERENCES

There are no appeals or interferences related to the present application.

III. STATUS OF CLAIMS

Pending claims: 14-76 and 93-110.

Canceled claims: 1-13 and 77-92.

Allowed claims: 39-50.

Rejected claims: 14, 15, 17-24, 26-32, 34-37, 51-62, 64-75, 93, 95, 98-104, and 107-110.

Objected to claims: 16, 25, 33, 38, 63, 76, 94, 96, 97, 105, and 106.

Appellants' understanding of the foregoing status is informed, in part, by the clarification set forth in the Advisory Action dated May 20, 2005 ("Advisory Action"). Specifically, the PTO objected to those claims identified above for depending from rejected base claims. Advisory Action at page 1. Appellants kindly point out, however, that claims 105 and 106, to which the PTO objects, both depend from allowed claim 39, and not from any rejected claim. Consequently, claims 105 and 106 should also be allowed.

IV. STATUS OF AMENDMENTS

An after-final response was filed on April 18, 2005, but that response did not amend any of the claims. Appellants last amended the claims in their response dated August 19, 2004.

V. SUMMARY OF CLAIMED SUBJECT MATTER

The claimed invention is directed to bioadhesive compositions of nanoparticulate active agents and methods of using the same. The active agent particles, or liquid droplets comprising active agent, have an effective average particle size of less than about 4 microns. *See* specification at page 3, lines 13-15.

In a first embodiment ("First Embodiment"), the composition comprises: (a) a water-soluble or poorly water-soluble crystalline nanoparticulate active agent; and (b) at least one cationic primary

surface stabilizer (claims 14-26, 98, 101, and 102). *See id.* at page 3, lines 10-21. These compositions do not comprise a phospholipid.

In a second embodiment ("Second Embodiment"), the composition comprises: (a) water-soluble or poorly water-soluble active agent particles which are in a liquid state at or near room temperature; and (b) at least one cationic primary surface stabilizer, wherein the active agent particles are dispersed in a liquid medium in which they are poorly soluble (claims 27-50 and 103-106). *See id.* at page 3, line 22 through page 4, line 6.

In a third embodiment ("Third Embodiment"), the composition comprises: (a) active agent dissolved or dispersed in liquid droplets of a water-soluble or poorly water-soluble liquid; and (b) at least one cationic primary surface stabilizer adsorbed to the surface of the liquid droplets, wherein the liquid droplets are dispersed in a liquid medium in which they are poorly soluble (claims 51-76, 99, and 107-110). *See id.* at page 4, lines 7-19.

Finally, the invention encompasses methods of using the nanoparticulate active agent compositions of the invention. First and second methods encompass applying a nanoparticulate active agent formulation to a biological surface. *See id.* at page 6, lines 7-11. The active agent particles of the first method can be in a semi-crystalline state, an amorphous state, a mixture of crystalline and semi-crystalline, a mixture of crystalline and amorphous, or a mixture of crystalline, semi-crystalline, and amorphous (claim 93 and 94). *See id.* at page 15, lines 5-7.

The active agent particles of the second method are in a crystalline state (claims 95 and 96). *See id.* at page 15, line 6. The compositions of these two methods do not comprise a phospholipid. A third method encompasses applying a nanoparticulate composition comprising agriculturally active agent particles to plant tissue. *See id.* at page 6, lines 12-14.

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

Three issues remain after the Final Office Action dated November 18, 2004 ("Final Office Action"):

A. Issue 1: Whether claims 14, 15, 17-21, 23, 24, 25, 27, 29-32, 34, 36, 37, 51, 52, 54-59, 61, 62, 64, 66-72, 74, 75, 93, 95, 101-104, and 107-110 are unpatentable under 35 U.S.C. § 103(a) over U.S. Patent No. 6,177,103 to Pace et al. ("Pace");

B. Issue 2: Whether claims 22, 28, 35, 53, 60, 65, and 73 are unpatentable under 35 U.S.C. § 103(a) over the combination of Pace and U.S. Patent No. 5,145,684 to Liversidge et al. ("Liversidge"); and

C. Issue 3: Whether claims 98-100 are unpatentable under 35 U.S.C. § 103(a) over the combination of Pace and U.S. Patent No. 5,891,420 to Cutie ("Cutie").

A listing of the claims on appeal is presented in Appendix A.

VII. ARGUMENT

A. Issue 1: The Claimed Invention is Patentable Over Pace Because Pace Fails to Teach at Least One Claim Element for Each of Appellants' Claimed Compositions and Methods of Use

Claims 14, 15, 17-21, 23, 24, 25, 27, 29-32, 34, 36, 37, 51, 52, 54-59, 61, 62, 64, 66-72, 74, 75, 93, 95, 101-104, and 107-110 were rejected by the PTO as being allegedly unpatentable under 35 U.S.C. § 103(a) over Pace. *See* Final Office Action at page 2.

The claimed nanoparticulate drug compositions and methods of using the same are patentable over Pace because the PTO failed to establish a *prima facie* case of obviousness. Specifically, Pace

does not teach or suggest the specific nanoparticulate compositions identified as Embodiments One, Two, and Three, *supra*, as claimed.

> To establish a *prima facie* case of obviousness, three basic criteria must be met. First, the prior art reference (or references when combined) must teach or suggest all of the claim limitations. Second, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Finally, there must be a reasonable expectation of success.

See MPEP § 2143 (Rev. 2, May 2004). Specifically, it is incumbent upon the PTO to demonstrate that Pace teaches or would have suggested the claimed nanoparticulate compositions requiring, in relevant part, (1) crystalline active agent particles (Embodiment One, rejected claims 14, 15, 17-21, 23-25, 95, 101, 102); (2) active agent particles that are in a liquid state and that are poorly water-soluble (Embodiment Two, rejected claims 27, 29-32, 34, 36, 37, 103, 104); or (3) an active agent that is dissolved or dispersed in liquid droplets of a water-soluble or poorly water-soluble liquid, where those droplets are dispersed in a liquid medium in which the droplets are poorly soluble (Embodiment Three, rejected claims 51, 52, 54-59, 61, 62, 64, 66-72, 74, 75, and 107-110). Additionally, the PTO must demonstrate that Pace teaches or would have suggested a method of applying a nanoparticulate formulation to a biological surface as claimed in rejected claim 93. The PTO's analysis falls short of satisfying this requirement

1. Summary of Pace

Pace teaches submicron particles of a water-insoluble or water poorly soluble compound, particularly a drug, prepared by simultaneously stabilizing a microparticulate suspension of the compounds with surface modifier molecules and rapidly expanding the suspensions into an aqueous medium from a compressed solution of the compound and surface modifiers in a liquefied gas. *See* Abstract and col. 5, lines 14-21, of Pace. Examples of suitable surface modifiers include cationic surfactants.

The objective of Pace is to "develop a process with high productivity based on the use of liquefied gas solvents, including supercritical fluid technology, that yields surface modifier stabilized suspensions of water insoluble drugs . . ." Pace at col. 4, lines 63-67.

Furthermore, insofar as Pace teaches anything about the morphology of the disclosed compounds, the reference teaches compositions comprising amorphous drugs. Thus, Pace states that "[a] rapid intimate contact between the surface modifier and the newly formed particle substantially inhibits the crystal growth of the newly formed particle." *See e.g.*, col. 4, lines 38-40 of Pace. In addition, the method of Pace requires "very fast precipitation" (col. 4, line 31) and "very rapid precipitation" (col, 4, line 33). Very rapid precipitation is the preferred way to produce, and is widely recognized to result in amorphous particles primarily because the phase transition (from solution to solid) is too rapid for the active agent molecules to organize themselves into a coherent crystal lattice. *See* Appellants' Response dated April 18, 2005 at pages 21-22 and Reverchon et al. submitted therewith (Appendix B).

2. Pace Does Not Teach or Suggest Crystalline Active Agents, as Required by Rejected Claims 14, 15, 17-21, 23, 24, 25, 95, 101, 102

Pace fails to support the rejection because, in the first instance, the reference admittedly "is silent as to the teaching of the [claimed] crystalline particles", Final Office Action at page 4, and it therefore does not teach all of the claim limitations of Embodiment One (claims 14-26, 95, 96, 98, 101, and 102). In the second instance, such silence, combined with Pace's affirmative teaching of amorphous active agent particles, vitiates the PTO's argument that the routineer would nonetheless have been motivated to make the claimed crystalline active agent nanoparticulate composition.

The PTO's finding of obviousness as to this embodiment turns entirely and impermissibly upon the skill of the ordinary artisan who, through "routine experimentation", would have somehow made the claimed composition comprising crystalline active agents despite the absence of any such

suggestion by Pace. Based on nothing more than blurring the difference between crystalline active agents as claimed and the amorphous compounds taught by Pace, the PTO required Appellants to furnish "any unexpected result over the particles taught by Pace" to rebut the PTO's unsupported allegation of "no criticality in the crystalline particle [*sic*] being claimed." Final Office Action at page 4. *See also* Advisory Action at page 3.

a. The PTO Did Not Identify A Proper Motivation to Modify Pace

Substantial authority flatly proscribes the PTO's rationale underlying this ground for rejection. "Skill in the art does not act as a bridge over gaps in substantive presentation of an obviousness case, but instead supplies the primary guarantee of objectivity in the process." *Al-Site Corp. v. VSI Int'l Inc.*, 174 F.3d 1308, 1324, 50 USPQ2d 1161, 1171 (Fed. Cir. 1999). *See also Ex parte Levengood*, 28 USPQ2d 1300 (Bd. App. App. & Inter. 1993); *In re Kotzab*, 217 F.3d 1365, 1371, 55 USPQ2d 1313, 1318 (Fed. Cir. 2000). Rather, "[t]he factual inquiry whether to combine [or modify] references must be thorough and searching. It must be based on objective evidence of record." *See In re Sang-Su Lee*, 277 F.3d 1338, 1343, 61 USPQ2d 1430, 1433 (Fed. Cir. 2002) (quoting *McGinley v. Franklin Sports, Inc.*, 262 F.3d 1339, 1351-52, 60 USPQ2d 1001, 1008 (Fed. Cir. 2001) (internal quotes and citation omitted).

The PTO advanced no motivation whatsoever, either in "the nature of the problem to be solved, the teachings of the prior art, [or] the knowledge of persons of ordinary skill in the art", *In re Rouffet*, 149 F.3d 1350, 1357, 47 USPQ2d 1453 (Fed. Cir. 1998), whereby a person of ordinary skill in the art would have modified the amorphous compound of Pace so as to employ the crystalline active agent as presently claimed. Instead, the PTO settled upon the notion that "routine experimentation [would] determine a suitable size and shape of the particle to obtain the claimed invention." *See* Advisory Action at page 3.

Absent any motivation that the PTO must reveal by a "thorough and searching" factual inquiry, *In re Sang-Su Lee*, 277 F.3d at 1343, 61 USPQ2d at 1433, the person of ordinary skill would have been left simply with the uncontested fact that Pace fails to teach or suggest the requisite crystalline active agent. Supposing that ordinary skill in the art is an additional fact to be relied upon does not manufacture motivation. Because the PTO did not identify a motivation or suggestion to modify Pace to arrive at Appellants' invention, the PTO has not established a *prima facie* case of obviousness.

b. The PTO Applied an Improper Legal Standard in Making the Determination of Obviousness

The PTO did not establish a *prima facie* case of obviousness for the additional reason that, in the first instance, the PTO effectively ignored the crystalline active agent element of the invention on the grounds that "there's [*sic*] no criticality in the crystalline particle being claimed", Advisory Action at page 3, and, in the second instance, then required Appellants to demonstrate "criticality." Final Office Action at page 4. *See also* Advisory Action at page 3. The PTO in so doing invoked an incorrect legal standard.

It is black letter law that "[t]he examiner bears the burden of establishing a *prima facie* case of obviousness." *In re Deuel*, 51 F. 3d 1552, 1557, 34 USPQ2d 1210, 1217 (Fed. Cir. 1995). "*Only if* that burden is met, does the burden of coming forward with evidence or argument shift to the applicant." *In re Rijckaert*, 9 F. 3d 1531, 1532, 28 USPQ2d 1955, 1956 (Fed. Cir. 1993) (emphasis supplied). In this analysis, "[a]ll words in a claim must be considered in judging the patentability of that claim against the prior art." *In re Wilson*, 424 F. 2d 1382, 1385 (CCPA 1970).

Applying these principles to the present scenario, the PTO is therefore obligated to treat the crystalline active agent element as a feature of Appellants' invention and then show why a person of ordinary skill in the art would have considered that element, in the context of Appellants' invention,

to have been obvious over Pace. Instead, as discussed above, the PTO considered it to be a foregone conclusion without elaboration that the claimed crystalline active agent would have resulted from "routine experimentation." Advisory Action at page 3.

Because the PTO did not meet its burden of establishing a *prima facie* case of obviousness, and because Pace does not teach or suggest nanoparticulate compositions comprising crystalline active agents, the claims directed to this embodiment are patentable over the cited combination.

c. Pace Teaches *Away* From Compositions Comprising a Crystalline Active Agent

As noted above, Pace teaches that the described method results in amorphous active agent particles. Thus, at the time of Appellants' claimed invention, one of skill in the art reading Pace would not have been motivated to modify the Pace teaching in an attempt to make compositions comprising crystalline active agent particles.

3. Pace Does Not Teach or Suggest Active Agent Particles Which Are In a Liquid State, as Required by Rejected Claims 27, 29-32, 34, 36, 37, 103, 104

Pace additionally fails to support the rejection because the reference is limited to particles of solid active agents, and it therefore does not teach or suggest "poorly water-soluble active agent particles which are in a liquid state at or near room temperature" as required by the claims of Embodiment Two (claims 27-50 and 103-106; emphasis added). As best as Appellants can understand the Advisory Action, the PTO has maintained this ground for rejection because "[t]he claim requires the active agent particles be [*sic*] dispersed in a liquid medium in which they are poorly soluble." Advisory Action at page 3.

The PTO apparently missed the point entirely: Pace does not teach or suggest *any* particles of an active agent that are in a liquid state. Absent such disclosure or suggestion, the claims clearly distinguish over Pace.

Inconsistent, too, with this rejection is the PTO's allowing claims 39-50, which differ from the rejected claims of Embodiment 2 only by virtue of their reciting "water-soluble active agent particles." The distinguishing claim feature with respect to Pace, however, is not necessarily the water (in)solubility of the particles, but rather the liquid state of those particles. Thus, Pace does not teach or suggest nanoparticulate compositions comprising active agent particles in a liquid state, and the claims of Embodiment Two therefore are patentable over Pace.

4. Pace Does Not Teach or Suggest Active Agents That Are Dissolved or Dispersed in Liquid Droplets, as Required by Rejected Claims 51, 52, 54-59, 61, 62, 64, 66-72, 74, 75, and 107-110

Pace also does not support the rejection as to the claims directed to Embodiment Three (rejected claims 51, 52, 54-59, 61, 62, 64, 66-72, 74, 75, and 107-110). This is so because Pace fails to teach or suggest an active agent that is dissolved or dispersed in a liquid medium that is poorly water-soluble (*e.g.*, claims 51-63, 107, and 108) or water-soluble (*e.g.*, claims 64-76, 109, and 110).

a. Pace Does Not Teach or Suggest an Active Agent Dissolved or Dispersed in Droplets of a Poorly Water-Soluble Liquid, as Required by Claims 51-63, 107, and 108

Pace fails to teach or suggest an active agent that is dissolved or dispersed in droplets of a liquid that is *poorly water-soluble*, as required by claims 51-63, 107, and 108. The PTO cited col. 5, lines 43-47, and Example 1 as support for the conclusion that Pace teaches the claimed invention. However, these passages of Pace do not teach or suggest that the claimed droplets are formed, nor does any other passage of Pace teach this aspect of the claimed invention.

Instead, Pace teaches that a water insoluble substance may be dissolved or dispersed in a liquefied gas, and then expanded into an aqueous dispersion. *See* Pace at col. 5, lines 14-21 and lines 33-38. There is no teaching or suggestion that such expansion results in the formation of droplets containing the water insoluble substance, which droplets are dispersed in the aqueous medium.

b. Pace Does Not Teach or Suggest an Active Agent Dispersed in Droplets of a Water-Soluble Liquid, as Required by Claims 64-76, 109, and 110

In addition, Pace fails to teach active agents that are dispersed in liquid droplets of *a water soluble liquid*, as required by claims 64-76, 109, and 110. As previously stated, Pace describes making particulate compositions using liquefied gas solvents, including supercritical fluids. Examples of useful liquefied gases are described at col. 6, lines 6-33, of Pace. None of the liquefied gasses are "water soluble," because in the process of Pace, the compressed solution of the compound and surface modifiers in a liquefied gas are expanded into an aqueous medium. Such expansion is not possible if the liquefied gas is soluble in water (*i.e.*, an aqueous medium). Accordingly, Pace fails to teach active agents dispersed in liquid droplets of *a water soluble liquid*, as required by the claimed invention.

c. Pace Fails to Teach or Suggest Droplets Having a Surface Modifier Adsorbed to the Surface Thereof, as Required by Claims 51-76, 99, 100, and 107-110

Even if Pace prescribed the formation of droplets, a proposition that Appellants do not endorse, the droplets would not have surface modifiers adsorbed on the surfaces of the droplets as required by claims 51-76, 99, 100, and 107-110. This is because the surface modifiers taught by Pace "are chosen to be active at the compound-water interface" (Pace at col. 3, lines 63-67) and not at the liquid-liquid interface. Thus, the process as taught by Pace results in surface modifiers being adsorbed to particles of a compound and not to the surfaces of liquid droplets, as required by claims 51-76, 99, 100, and 107-110. In fact, Pace emphasizes that "[t]he principle feature of [Pace] is believed to be rapid attainment of intimate contact of the dissolved drug and the surface modifier . . ." Pace at col. 4, lines 30-33. Accordingly, Pace itself teaches against compositions having a surface modifier absorbed to the surface of droplets comprising active agent.

5. Pace Does Not Teach or Suggest A Method of Applying a Nanoparticulate Formulation to a Biological Surface as Required by Claim 93

Finally, Pace does not support the rejection for the additional reason that Pace is absolutely silent as to administering the recited nanoparticulate formulation to any biological surface as required by claim 93. In fact, at no time during the entire prosecution of this application has the PTO established that Pace teaches or suggests such a method. Absent a disclosure of all the claimed elements or at least a suggestion to modify Pace to arrive at the claimed method, Pace simply does not support a *prima facie* case of obviousness against claim 93.

* * *

For all of the foregoing reasons, the claimed invention is patentable over Pace. Accordingly, Appellants respectfully request the Board to reconsider and withdraw this ground for rejection.

B. Issue 2: The Claims Are Patentable Over the Combination of Pace and Liversidge

Claims 22, 28, 35, 53, 60, 65, and 73 were rejected by the Examiner as being allegedly are unpatentable under 35 U.S.C. § 103(a) over the combination of Pace and Liversidge. Pace and Liversidge, alone or in combination with each other, do not render obvious claims 22, 28, 35, 53, 60, 65, and 73. According to the PTO, "Pace does not expressly teach that the composition further comprises an excipient or that water is used as the dispersion medium. Liversidge teaches that such composition can further comprise a carrier (excipient) and that the dispersion medium can be water." Final Office Action at page 3. The PTO further elaborated that Liversidge was cited "solely for the teaching of the specific carrier . . ." Advisory Action at page 4.

1. Liversidge Fails to Remedy the Deficiencies of Pace

Liversidge does not remedy the deficiencies of Pace that are discussed above. A combination of references that fails to teach all claim limitations cannot form a *prima facie* case of obviousness. *See In re Royka* , 490 F.2d 981, 180 USPQ 580 (CCPA 1974). Here, neither Pace nor Liversidge

teach compositions comprising particles that are *liquids* at or near room temperature, as required by claims 28 and 35; rather, these references teach only *solid* particles. In addition, neither Pace nor Liversidge teach active agents that are dispersed or dissolved in liquid droplets of a water soluble or poorly water soluble liquid, as required by claims 53, 60, 65, and 73.

2. Claims 22, 28, 35, 53, 60, 65, and 73 are Patentable Over Pace in View of Liversidge, as There is no Motivation to Combine Pace and Liversidge

Moreover, the PTO identified no motivation whereby a person of ordinary skill would have combined the teachings of Pace and Liversidge. In fact, Pace and Liversidge teach away from the claimed invention. Pace teaches a process for preparing compositions comprising amorphous compounds. *Supra*. For example, the method of Pace requires "very fast precipitation" (col. 4, line 31) and "very rapid precipitation" (col. 4, line 33). A skilled artisan understands that "very rapid precipitation" results in an amorphous compound.

In contrast, Liversidge teaches milling a *crystalline* drug. *See* Liversidge at col. 3, lines 32-37. Liversidge goes so far as to distinguish milling from other methods that result in amorphous drugs. *See id.* ("The drug substance exists as a discrete crystalline phase. The crystalline phase differs from a non-crystalline or amorphous phase which results from a precipitation technique . . ."). Thus, Pace and Liversidge are diametrically opposed because the former teaches a method for preparing amorphous compounds and the latter teaches a method of preparing crystalline compounds.

Accordingly, there is no motivation to combine the references. For at least these reasons, claims 22, 28, 35, 53, 60, 65, and 73 are patentable over this combination of references. Appellants respectfully request the Board to withdraw this ground for rejection.

C. Issue 3: The Claims Are Patentable Over the Combination of Pace and Cutie

Claims 98-100 were rejected by the Examiner as being allegedly unpatentable under 35 U.S.C. § 103(a) over the combination of Pace and Cutie. Pace and Cutie, alone or in combination

with each other, do not render obvious the subject matter of claims 98-100. Appellants understand that the PTO cited Cutie "solely for the teaching of the specific active agent."

This reference, as with Liversidge, does not address the deficiencies of Pace that Appellants discuss above. Even if Pace and Cutie could be combined in a manner that addressed all of the features of the claims, a person of ordinary skill would not have been motivated to make such combination because the compositions of Cutie specifically exclude surfactants (*i.e.,* surface stabilizers), which are required components of the claimed invention. *See* Cutie at col. 3, lines 41-42.

For at least these reasons, Pace and Cutie do not teach or suggest the claimed invention. Accordingly, Appellants respectfully request the Board to withdraw this ground for rejection.

VIII. CONCLUSION

Pace, alone or in combination with Liversidge or Cutie, do not teach or suggest the claimed nanoparticulate compositions and methods. Pace fails to teach nanoparticulate compositions comprising active agents that are crystalline, in a liquid state at or near room temperature, or are dispersed or dissolved in water soluble or water-insoluble liquid droplets. The secondary references of Liversidge and Cutie fail to address these shortcomings. For all of these reasons, the PTO has not established a *prima facie* case of obviousness. Accordingly, Appellants respectfully urge the Board to reconsider and reverse the outstanding rejections of the claims.

Respectfully submitted,

Date August 16, 2005

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APPENDIX A

APPENDIX A: CLAIMS ON APPEAL

Claims 1-13 (Canceled).

14. (Previously Presented) A stable bioadhesive nanoparticulate composition which adsorbs to a biological surface and which comprises:

(a) active agent particles in a crystalline state, wherein the active agent particles have an effective average particle size of less than about 4000 nm;

and

(b) adsorbed to the surface thereof at least one cationic surface stabilizer selected from the group consisting of a polymer, a biopolymer, a polysaccharide, a cellulosic, an alginate, and a nonpolymeric compound,

wherein:

(i) the nonpolymeric compound is not benzalkonium chloride; and

(ii) the composition does not comprise a phospholipid.

15. (Original) The composition of claim 14 having benzalkonium chloride as a secondary surface stabilizer.

16. (Original) The composition of claim 14, wherein the surface stabilizer is selected from the group consisting of polymethylmethacrylate trimethylammonium bromide, polyvinylpyrrolidone-2-dimethylaminoethyl methacrylate dimethyl sulfate, and hexadecyltrimethyl ammonium bromide.

17. (Original) The composition of claim 14, wherein the active agent is selected from the group consisting of a poorly water-soluble active agent and a water-soluble active agent.

18. (Original) The composition of claim 14, wherein the active agent is selected from the group consisting of a drug, vitamin, herb, cosmetic agent, coloring agent, flavor agent, fragrance agent, sunscreen, moisturizer, deodorant, food product, hair conditioner agent, hair dye, hair spray agent, hair cosmetic agent, hair cleanser agent, depilatory agent, insecticide, fertilizer, pesticide, herbicide, germicide, and plant growth regulating agent.

19. (Original) The composition of claim 18, wherein the drug is selected from the group consisting of proteins, peptides, nutriceuticals, anti-obesity agents, corticosteroids, elastase inhibitors, analgesics, anti-fungals, oncology therapies, anti-emetics, analgesics, cardiovascular agents, anti-

inflammatory agents, anthelmintics, anti-arrhythmic agents, antibiotics, anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents, anxiolytic sedatives, astringents, beta-adrenoceptor blocking agents, blood products and substitutes, cardiac inotropic agents, contrast media, cough suppressants, diagnostic agents, diagnostic imaging agents, diuretics, dopaminergics, haemostatics, immunological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin and biphosphonates, prostaglandins, radio- pharmaceuticals, sex hormones, anti-allergic agents, stimulants and anoretics, sympathomimetics, thyroid agents, vasodilators, xanthines, acne medication, alpha-hydroxy formulations, cystic-fibrosis therapies, asthma therapies, emphysema therapies, respiratory distress syndrome therapies, chronic bronchitis therapies, chronic obstructive pulmonary disease therapies, organ-transplant rejection therapies, therapies for tuberculosis and other infections of the lung, and respiratory illness therapies associated with acquired immune deficiency syndrome.

20. (Original) The composition of claim 14, wherein the composition is formulated for administration selected from the group consisting of vaginal, ocular, nasal, buccal, oral, colonic, topical, and subcutaneous administration.

21. (Original) The composition of claim 14, wherein the effective average particle size of the agent particles is selected from the group consisting of less than about 3500 nm, less than about 3000 nm, less than about 2500 nm, less than about 2000 nm, less than about 1500 nm, less than about 1000 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 100 nm, and less than about 50 nm.

22. (Original) The composition of claim 14, wherein the composition further comprises one or more pharmaceutically acceptable excipients.

23. (Original) The composition of claim 14, wherein the particles are present in an amount of about 99.99 to 0.01(w/w) based on the total weight of the composition.

24. (Original) The composition of claim 14, wherein the surface stabilizer is present in an amount of about 0.001 to about 99.999% (w/w) based on the total weight of the composition.

25. (Original) The composition of claim 14, wherein the composition adsorbs to a biological surface selected from the group consisting of an insect, teeth, bone, nails, chitin, feathers, scales, mucous, skin, hair, and plant tissue.

26. (Original) The composition of claim 14 in a dry powder form.

27. (Original) A stable bioadhesive nanoparticulate composition comprising:

(a) poorly water-soluble active agent particles which are in a liquid state at or near room temperature; and

(b) adsorbed to the surface thereof at least one cationic surface stabilizer,

wherein:

(i) the active agent particles are dispersed in a liquid medium in which they are poorly soluble;

(ii) the active agent particles have an effective average particle size of less than about 4000 nm; and

(iii) the nanoparticulate composition adsorbs to a biological surface.

28. (Original) The composition of claim 27, wherein the dispersion medium is water.

29. (Original) The composition of claim 27, wherein the active agent is selected from the group consisting of a drug, vitamin, herb, cosmetic agent, coloring agent, flavor agent, fragrance agent, sunscreen, moisturizer, deodorant, food product, hair conditioner agent, hair dye, hair spray agent, hair cosmetic agent, hair cleanser agent, depilatory agent, insecticide, fertilizer, pesticide, herbicide, germicide, and plant growth regulating agent.

30. (Original) The composition of claim 29, wherein the drug is selected from the group consisting of proteins, peptides, nutriceuticals, anti-obesity agents, elastase inhibitors, analgesics, anti-fungals, oncology therapies, anti-emetics, analgesics, cardiovascular agents, anti-inflammatory agents, anthelmintics, anti-arrhythmic agents, antibiotics, anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents, anxiolytic sedatives, astringents, beta-adrenoceptor blocking agents, blood products and substitutes, cardiac inotropic agents, contrast media, corticosteroids, cough suppressants, diagnostic agents, diagnostic imaging agents, diuretics, dopaminergics, haemostatics, immunological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin and biphosphonates, prostaglandins, radio- pharmaceuticals, sex hormones, anti-allergic agents, stimulants and anoretics, sympathomimetics, thyroid agents, vasodilators, xanthines, cystic-fibrosis therapies, asthma therapies, emphysema therapies, respiratory distress syndrome therapies, chronic bronchitis therapies, chronic obstructive pulmonary disease therapies, organ-transplant rejection therapies, therapies for tuberculosis and other infections of the lung, and respiratory illness therapies associated with acquired immune deficiency syndrome.

31. (Original) The composition of claim 27, wherein the composition is formulated for administration selected from the group consisting of vaginal, ocular, nasal, buccal, oral, colonic, topical, and subcutaneous administration.

32. (Original) The composition of claim 27, wherein the at least one cationic surface stabilizer is selected from the group consisting of a polymer, a biopolymer, a polysaccharide, a cellulosic, an alginate, a nonpolymeric compound, and a phospholipid.

33. (Original) The composition of claim 27, wherein the surface stabilizer is selected from the group consisting of benzalkonium chloride, polymethylmethacrylate trimethylammonium bromide, polyvinylpyrrolidone-2-dimethylaminoethyl methacrylate dimethyl sulfate, and hexadecyltrimethyl ammonium bromide.

34. (Original) The composition of claim 27, wherein the effective average particle size of the agent particles is selected from the group consisting of less than about 3500 nm, less than 3000 nm less than 2500 nm, less than 2000 nm, less than about 1500 nm, less than about 1000 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 100 nm, and less than about 50 nm.

35. (Original) The composition of claim 27, wherein the composition further comprises one or more pharmaceutically acceptable excipients.

36. (Original) The composition of claim 27, wherein the particles are present in an amount of about 99.99 to 0.01% (w/w) based on the total weight of the composition.

37. (Original) The composition of claim 27, wherein the surface stabilizer is present in an amount of about 0.001 to about 99.999% (w/w).

38. (Original) The composition of claim 27, wherein the composition adsorbs to a biological surface selected from the group consisting of an insect, teeth, bone, nails, chitin, feathers, scales, mucous, skin, hair, and plant tissue.

Claims 39-50 are allowed and are therefore not on appeal.

51. (Original) A stable bioadhesive nanoparticulate composition comprising:

(a) active agent dissolved or dispersed in liquid droplets of a poorly water-soluble liquid; and

(b) adsorbed to the surface of the liquid droplets at least one cationic surface stabilizer,

wherein:

(i) the liquid droplets comprising active agent are dispersed in a liquid medium in which they are poorly soluble;

(ii) the liquid droplets comprising active agent have an effective average particle size of less than about 4000 nm; and

(iii) the nanoparticulate composition adsorbs to a biological surface.

52. (Original) The composition of claim 51, wherein the poorly water-soluble liquid in which the active agent is dissolved is selected from the group consisting of mineral oil, vegetable oils, and a hydrocarbon.

53. (Original) The composition of claim 51, wherein the liquid droplets comprising active agent are dispersed in water.

54. (Original) The composition of claim 51, wherein the active agent is selected from the group consisting of a drug, vitamin, herb, cosmetic agent, coloring agent, flavor agent, fragrance agent, sunscreen, moisturizer, deodorant, food product, hair conditioner agent, hair dye, hair spray agent, hair cosmetic agent, hair cleanser agent, depilatory agent, insecticide, fertilizer, pesticide, herbicide, germicide, and plant growth regulating agent.

55. (Original) The composition of claim 51, wherein the drug is selected from the group consisting of proteins, peptides, nutriceuticals, anti-obesity agents, elastase inhibitors, analgesics, anti-fungals, oncology therapies, anti-emetics, analgesics, cardiovascular agents, anti-inflammatory agents, anthelmintics, anti-arrhythmic agents, antibiotics, anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents, anxiolytic sedatives, astringents, beta-adrenoceptor blocking agents, blood products and substitutes, cardiac inotropic agents, contrast media, corticosteroids, cough suppressants, diagnostic agents, diagnostic imaging agents, diuretics, dopaminergics, haemostatics, immunological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin and

biphosphonates, prostaglandins, radio- pharmaceuticals, sex hormones, anti-allergic agents, stimulants and anoretics, sympathomimetics, thyroid agents, vasodilators, xanthines, cystic-fibrosis therapies, asthma therapies, emphysema therapies, respiratory distress syndrome therapies, chronic bronchitis therapies, chronic obstructive pulmonary disease therapies, organ-transplant rejection therapies, therapies for tuberculosis and other infections of the lung, and respiratory illness therapies associated with acquired immune deficiency syndrome.

56. (Original) The composition of claim 51, wherein the composition is formulated for administration selected from the group consisting of vaginal, ocular, nasal, buccal, oral, colonic, topical, and subcutaneous administration.

57. (Original) The composition of claim 51, wherein the at least one cationic surface stabilizer is selected from the group consisting of a polymer, a biopolymer, a polysaccharide, a cellulosic, an alginate, a nonpolymeric compound, and a phospholipid.

58. (Original) The composition of claim 51, wherein the surface stabilizer is selected from the group consisting of benzalkonium chloride, polymethylmethacrylate trimethylammonium bromide, polyvinylpyrrolidone-2-dimethylaminoethyl methacrylate dimethyl sulfate, and hexadecyltrimethyl ammonium bromide.

59. (Original) The composition of claim 51, wherein the effective average particle size of the liquid droplets comprising active agent is selected from the group consisting of less than about 3500 nm, less than about 3000 nm, less than about 2500 nm, less than about 2000 nm less than about 1500 nm, less than about 1000 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 100 nm, and less than about 50 nm.

60. (Original) The composition of claim 51, wherein the composition further comprises one or more pharmaceutically acceptable excipients.

61. (Original) The composition of claim 51, wherein the active agent particles are present in an amount of about 99.99 to 0.01% (w/w).

62. (Original) The composition of claim 51, wherein the surface stabilizer is present in an amount of about 0.001 to about 99.999% (w/w).

63. (Original) The composition of claim 51, wherein the biological surface is selected from the group consisting of an insect, teeth, bone, nails, chitin, feathers, scales, mucous, skin, hair, and plant tissue.

64. (Original) A stable bioadhesive nanoparticulate composition comprising:

(a) active agent dissolved or dispersed in liquid droplets of a water-soluble liquid; and

(b) adsorbed to the surface of the liquid droplets at least one cationic surface stabilizer,

wherein:

(i) the liquid droplets comprising active agent are dispersed in a liquid medium in which they are poorly soluble;

(ii) the liquid droplets comprising active agent have an effective average particle size of less than about 4000 nm; and

(iii) the nanoparticulate composition adsorbs to a biological surface.

65. (Original) The composition of claim 64, wherein the poorly water-soluble liquid in which the active agent is dissolved is water.

66. (Original) The composition of claim 64, wherein the liquid droplets comprising active agent are dispersed in a liquid medium selected from the group consisting of mineral oil, vegetable oils, and a hydrocarbon.

67. (Original) The composition of claim 64, wherein the active agent is selected from the group consisting of a drug, vitamin, herb, cosmetic agent, coloring agent, flavor agent, fragrance agent, sunscreen, moisturizer, deodorant, food product, hair conditioner agent, hair dye, hair spray agent, hair cosmetic agent, hair cleanser agent, depilatory agent, insecticide, fertilizer, pesticide, herbicide, germicide, and plant growth regulating agent.

68. (Original) The composition of claim 64, wherein the drug is selected from the group consisting of proteins, peptides, nutriceuticals, anti-obesity agents, elastase inhibitors, analgesics, anti-fungals, oncology therapies, anti-emetics, analgesics, cardiovascular agents, anti-inflammatory agents, anthelmintics, anti-arrhythmic agents, antibiotics, anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents, anxiolytic sedatives, astringents, beta-adrenoceptor blocking agents, blood products and substitutes, cardiac inotropic agents, contrast media, corticosteroids, cough suppressants, diagnostic

agents, diagnostic imaging agents, diuretics, dopaminergics, haemostatics, immunological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin and biphosphonates, prostaglandins, radio- pharmaceuticals, sex hormones, anti-allergic agents, stimulants and anoretics, sympathomimetics, thyroid agents, vasodilators, xanthines, cystic-fibrosis therapies, asthma therapies, emphysema therapies, respiratory distress syndrome therapies, chronic bronchitis therapies, chronic obstructive pulmonary disease therapies, organ-transplant rejection therapies, therapies for tuberculosis and other infections of the lung, and respiratory illness therapies associated with acquired immune deficiency syndrome.

69. (Original) The composition of claim 64, wherein the composition is formulated for administration selected from the group consisting of vaginal, ocular, nasal, buccal, oral, colonic, topical, and subcutaneous administration.

70. (Original) The composition of claim 64, wherein the at least one cationic surface stabilizer is selected from the group consisting of a polymer, a biopolymer, a polysaccharide, a cellulosic, an alginate, a nonpolymeric compound, and a phospholipid.

71. (Original) The composition of claim 64, wherein the surface stabilizer is selected from the group consisting of benzalkonium chloride, polymethylmethacrylate trimethylammonium bromide, polyvinylpyrrolidone-2-dimethylaminoethyl methacrylate dimethyl sulfate, and hexadecyltrimethyl ammonium bromide.

72. (Original) The composition of claim 64, wherein the effective average particle size of the liquid droplets comprising active agent is selected from the group consisting of less than about 3500 nm, less than about 3000 nm, less than about 2500 nm, less than about 2000 nm less than about 1500 nm, less than about 1000 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 100 nm, and less than about 50 nm.

73. (Original) The composition of claim 64, wherein the composition further comprises one or more pharmaceutically acceptable excipients.

74. (Original) The composition of claim 64, wherein the active agent particles are present in an amount of about 99.99 to 0.01% (w/w).

75. (Original) The composition of claim 64, wherein the surface stabilizer is present in an amount of about 0.001 to about 99.999% (w/w).

76. (Original) The composition of claim 64, wherein the biological surface is selected from the group consisting of an insect, teeth, bone, nails, chitin, feathers, scales, mucous, skin, hair, and plant tissue.

Claims 77-92 (Canceled).

93. (Previously Amended) A method of applying a nanoparticulate formulation to a biological surface comprising administering to the biological surface in need of such application a formulation comprising:

(a) active agent particles in a semi-crystalline state, an amorphous state, a mixture of crystalline and semi-crystalline, a mixture of crystalline and amorphous, or a mixture of crystalline, semi-crystalline, and amorphous; and

(b) adsorbed to the surface thereof at least one cationic surface stabilizer,

wherein:

(i) the active agent particles have an effective average particle size of less than about 4000 nm,

(ii) the nanoparticulate composition adsorbs to the biological surface; and

(iii) the composition does not comprise a phospholipid.

94. (Original) The method of claim 93, wherein the biological surface is selected from the group consisting of an insect, teeth, bone, nails, chitin, feathers, scales, mucous, skin, hair, and plant tissue.

95. (Previously Amended) A method of applying a nanoparticulate formulation to a biological surface comprising administering to the biological surface in need of such application formulation comprising:

(a) active agent particles in a crystalline state; and

(b) adsorbed to the surface thereof at least one cationic surface stabilizer selected from the group consisting of a polymer, a biopolymer, a polysaccharide, a cellulosic, an alginate, and a nonpolymeric compound,

wherein:

(i) the nonpolymeric compound is not benzalkonium chloride; and

(ii) the composition does not comprise a phospholipid.

96. (Original) The method of claims 95, wherein the biological surface is selected from the group consisting of an insect, teeth, bone, nails, chitin, feathers, scales, mucous, skin, hair, and plant tissue.

97. (Original) A method of applying a nanoparticulate formulation to plant tissue comprising administering to the plant tissue in need of such application a formulation comprising:

(a) agriculturally active agent particles; and

(b) adsorbed to the surface thereof at least one cationic surface stabilizer,

(c) wherein the active agent particles have an effective average particle size of less than about 4000 nm, and wherein the nanoparticulate composition adsorbs to the plant tissue.

98. (Previously Presented) The composition of claim 14, wherein the active agent is selected from the group consisting of naproxen, cyclosporine, triamcinolone acetonide, and benzoic acid, 3,5-bis(acetylamino) 2,4,6-triodo-, 4-(ethyl-3-ethoxy-2-butenoate) ester.

99. (Previously Presented) The composition of claim 51, wherein the active agent is selected from the group consisting of naproxen, cyclosporine, triamcinolone acetonide, and benzoic acid, 3,5-bis(acetylamino) 2,4,6-triodo-, 4-(ethyl-3-ethoxy-2-butenoate) ester.

100. (Previously Presented) The composition of claim 64, wherein the active agent is selected from the group consisting of naproxen, cyclosporine, triamcinolone acetonide, and benzoic acid, 3,5-bis(acetylamino) 2,4,6-triodo-, 4-(ethyl-3-ethoxy-2-butenoate) ester.

101. (Previously Presented) The composition of claim 14, further comprising a secondary non-cationic surface stabilizer.

102. (Previously Presented) The composition of claim 14, wherein at least 70%, at least 90%, or at least about 95% of the active agent particles have a weight average particle size of less than about 4 microns.

103. (Previously Presented) The composition of claim 27, further comprising a secondary non-cationic surface stabilizer.

104. (Previously Presented) The composition of claim 27, wherein at least 70%, at least 90%, or at least about 95% of the active agent particles have a weight average particle size of less than about 4 microns.

105. (Previously Presented) The composition of claim 39, further comprising a secondary non-cationic surface stabilizer.

106. (Previously Presented) The composition of claim 39, wherein at least 70%, at least 90%, or at least about 95% of the active agent particles have a weight average particle size of less than about 4 microns.

107. (Previously Presented) The composition of claim 51, further comprising a secondary non-cationic surface stabilizer.

108. (Previously Presented) The composition of claim 51, wherein at least 70%, at least 90%, or at least about 95% of the liquid droplets comprising active agent have a weight average particle size of less than about 4 microns.

109. (Previously Presented) The composition of claim 64, further comprising a secondary non-cationic surface stabilizer.

110. (Previously Presented) The composition of claim 64, wherein at least 70%, at least 90%, or at least about 95% of the liquid droplets comprising active agent have a weight average particle size of less than about 4 microns.

APPENDIX B

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Rifampicin microparticles production by supercritical antisolvent precipitation

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Abstract

Semi-continuous supercritical antisolvent (SAS) precipitation has been used to produce Rifampicin micro- and nanoparticles with controlled particle size (PS) and particle size distribution (PSD). SAS experiments were performed using different liquid solvents. The best micronization results have been obtained using dimethyl sulfoxide (DMSO); using this solvent and operating at 40 °C, we obtained nanoparticles with mean diameters ranging from 0.4 to 1 μm at a pressure of 120 bar or more, and microparticles with mean diameters ranging from 2.5 to 5 μm at pressures between 90 and 110 bar. The morphology of Rifampicin precipitates was different too. Nanoparticles connected in small aggregates were obtained at pressures higher than 120 bar, whereas, spherical single microparticles were obtained operating at lower pressures. We also investigated the effect of the concentration of Rifampicin in the liquid solution on particles diameter: we observed that, increasing the liquid concentration, the mean PS increased and the PSD enlarged. XRD and HPLC analysis on treated Rifampicin showed that particles are amorphous and no degradation occurred as a consequence of supercritical processing. We attempted an explanation of the different morphologies observed considering the modification of the high pressure vapor–liquid equilibria of the ternary system Rifampicin–DMSO–CO_2 with respect to the behavior of the binary system DMSO–CO_2.

Keywords: Rifampicin; Supercritical fluids; Microparticles; Antisolvent

1. Introduction

The micronization based on the use of supercritical antisolvents (SAS) has been suggested during the last years in alternative to traditional liquid antisolvent processes. Supercritical fluids in this process substitute the liquid antisolvent to induce the precipitation of microparticles with controlled diameter and particle size distribution (PSD). This technique has been applied by various research groups to explosives, catalysts, superconductor precursors (Gallagher et al., 1992; Reverchon et al., 1998, 1999), polymers (Dixon et al., 1993), biopolymers (Yeo et al., 1993; Rever-

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chon et al., 2000a), and some pharmaceutical compounds (Reverchon and Della Porta, 1999; Shekunov and York, 2000; Reverchon et al., 2000b; Chattopadhyay and Gupta, 2001; Velaga et al., 2002).

Rifampicin is an antibiotic that is mainly used for the treatment of tuberculosis; but, it is also used in the therapy of the meningitis and in the infections of the biliary ways. It acts preventing DNA transcription in the cells, a process in which the genetic information of DNA are transcribed in form of RNA. In particular, it interacts with RNA polymerase holoenzyme, resulting in abortive initiation and extension of transcription (Cole, 1996; Singh et al., 2001). This kind of antibiotics can attack either bacteric cells or guest organism cells. For this reason, they can be toxic for the human organism too. Rifampicin has a reduced toxicity for the human specie since it stops only the bacterial RNA-polymerase, the enzyme that in the bacteria gives rise to DNA transcription. Rifampicin is practically insoluble in water, thus, its dissolution in biological liquids is particularly difficult; therefore, its micronization can have a great impact on the effectiveness and duration of therapy. The production of micro and nanoparticles of controlled size and PSD of this antibiotic can reduce the therapeutic dosage and can avoid the effects correlated to the toxicity of the drug. Aerosolized microparticles could also be used to target the delivery of Rifampicin to alveolar macrophages trying a different approach to tuberculosis therapy. Suitable micronized Rifampicin particles will deposit on the lung periphery, where they can be ingested by alveolar macrophages and dissolution will occur (O'Hara and Hickey, 2000).

Therefore, the aim of this study is to ascertain the feasibility of SAS processing for this antibiotic to prepare micronic and submicronic particles. The powder morphologies, the role of SAS process parameters and the influence of different liquid solvents on the particle size (PS) and PSD are also studied. A windowed precipitation vessel has also been used to analyze the interactions of the high pressure vapor–liquid equilibria (VLE) with the precipitation process.

2. Experimental apparatuses, materials, procedures and methods

2.1. Apparatuses

Our SAS apparatus consists of two High-Performance Liquid Chromatography (HPLC) pumps (Gilson, mod. 305) used to deliver the liquid solution and supercritical CO_2, respectively. A cylindrical vessel of 500 cm^3 I.V. (I.D. = 5 cm) is used as precipitation chamber. The liquid mixture is delivered to the precipitator as a rule through a 60 μm diameter stainless steel nozzle. Supercritical CO_2 is delivered through another inlet port located on the top of the chamber. Before entering the precipitator, CO_2 is heated at the process temperature. The precipitation vessel is electrically heated using thin band heaters (Watlow, mod. STB3J2J1). The pressure in the chamber is measured using a test gauge manometer (Salmoiraghi, mod. SC-3200) and regulated by a micrometering valve (Hoke, mod. 1315G4Y) located at the exit (bottom) of the chamber. This valve is heated by a cable heater (Watlow, mod. 62H24ASX) connected to a controller. A stainless steel frit (pore diameter of 0.1 micron) located at the bottom of the chamber is used to collect the produced powder. A second collection vessel located downstream the micrometering valve is used to recover the liquid solvent. A backpressure valve (Tescom, mod. 26-1723-44) regulates the pressure in this vessel. At the exit of the second vessel a rotameter (Matheson, mod. 605) and a dry test meter are used to measure the CO_2 flow rate and the total quantity of antisolvent delivered, respectively. More information on the SAS apparatus was published elsewhere (Reverchon et al., 1998).

The transparent SAS apparatus differs from the other one only for the precipitator (NWA, Germany) that consists of a stainless steel cylindrical vessel (375 cm^3 I.V.) with two quartz windows put along all the longitudinal section (Fig. 1). Therefore, it is possible to visually follow the macroscopic evolution of the precipitation process from the liquid jet break-up to the deposition of precipitated particles.

2.2. Materials

Rifampicin with a purity of 99.9%, dimethyl sulfoxide (DMSO), *N*-methyl 2-pyrrolidone (NMP), methyl alcohol (MeOH), ethyl acetate (EtAc) and dichloromethane (MeC) with a purity of 99.5% were supplied by Sigma–Aldrich (Italy). CO_2 (purity 99.9%) was purchased from SON (Naples, Italy). The approximate solubilities of Rifampicin in DMSO, NMP, MeOH, EtAc and MeC were measured at room temperature and are 120, 80, 60, 40 and 60 mg/ml, respectively. The untreated material was formed by irregular crystals with a mean PS ranging between 20 and 100 μm (Fig. 2). All materials were used as received.

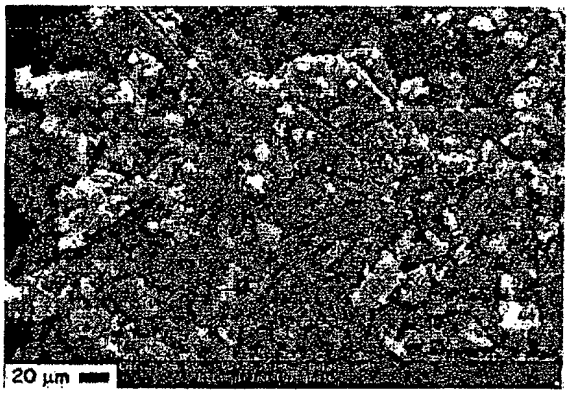

Fig. 2. SEM image of unprocessed Rifampicin.

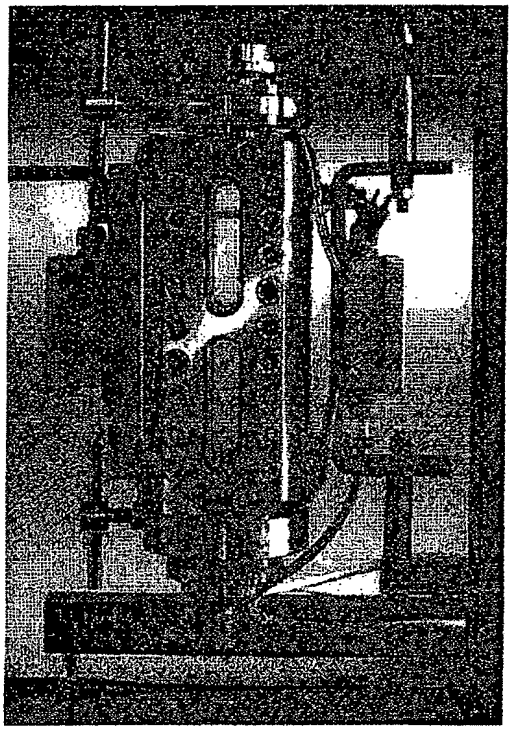

Fig. 1. Transparent SAS precipitator.

2.3. Experimental procedures

A SAS experiment begins by delivering supercritical CO_2 to the precipitation chamber until the desired pressure is reached. The antisolvent steady flow is then established. Then, pure solvent is sent through the nozzle to the chamber with the aim of obtaining steady state composition conditions during the solute precipitation. At this point, the flow of the liquid solvent is stopped and the liquid solution is delivered through the nozzle. The experiment ends when the delivery of the liquid solution to the chamber is interrupted. However, supercritical CO_2 continues to flow to wash the chamber for the residual content of liquid solubilized in the supercritical antisolvent. If the final purge with pure CO_2 is not done, solvent condenses during the depressurization and can solubilize the collected powder. More details were given elsewhere (Reverchon et al., 1998).

2.4. Analytical methods

Samples of the powder precipitated on the metallic frit were observed using a Scanning Electron Microscope (SEM) mod. LEO 420. SEM samples were covered with 250 Å of gold using a sputter coater (Agar mod. 108A). The PS and the PSD were measured using the Sigma Scan Pro software (Jandel Scientific) and about 1000 particles were considered in each calculation of PSD.

X-ray diffraction pattern (XRD) analysis was performed using a Philips PW 1050 XRD apparatus to ascertain if changes occurred in the crystal habit of Rifampicin as a consequence of the SAS process.

HPLC was performed to test if the SAS process modified the antibiotic. The HPLC system (Hewlett Packard series 1100) consisted of a micropump (mod. G1311A) and a UV detector set at 220 nm. The column used was a Zorbax RX C_8, 150 × 4.6 mm I.D., 5 μm PS. The mobile phase was 0.05 M potassium dihydrogen phosphate–acetonitrile (55:45 v/v) with a flow rate of 1 ml/min at ambient temperature. A standard solution of Rifampicin was prepared by dissolving 2 mg/ml of Rifampicin in MeOH (Lau et al., 1996).

3. Results and discussion

As a first step we tested SAS Rifampicin precipitation process from some solvents: DMSO, NMP, EtAc, MeOH and MeC using the stainless steel precipitator. The best results were obtained using DMSO since a powder formed by small particles precipitated in the collection vessel. In the other cases most of solute was recovered in the liquid collection vessel; i.e. the drug was partly extracted by the solution formed by the liquid solvent and supercritical CO_2 (Reverchon, 1999). In the experiments performed using NMP, MeOH and MeC, Rifampicin was precipitated in form of tightly networked nanoparticles, while using EtAc we mostly obtained some millimeters long needle-like crystals (Fig. 3). Based on these results, we decided to continue to test Rifampicin, using only DMSO as the liquid solvent.

The starting range of operating conditions used in our experiments was selected on the basis of our previous experiences on this process (Reverchon, 1999; Reverchon and Della Porta, 1999; Reverchon et al., 2000b). Thus, we performed a first set of experiments at a pressure of 120 bar. The other operating conditions were temperature 40 °C, 1 ml/min liquid solution flow rate. The ratio between CO_2 and liquid flow rates was set equal to 20 on a volumetric basis at the process

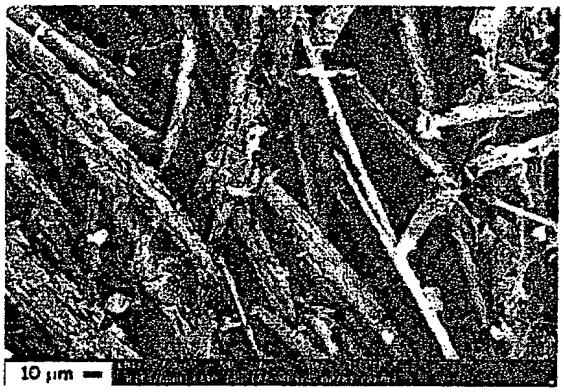

Fig. 3. SEM image of Rifampicin precipitated from EtAc at 145 bar, 40 °C and 5 mg/ml.

operating conditions. At these conditions we varied the concentration of the Rifampicin–DMSO solution from 10 to 70 mg/ml. SEM analysis of the powders precipitated in this set of experiments showed that Rifampicin was precipitated in form of nanoparticles coalescing in small groups. An example of this morphology is reported in Fig. 4.

Since the groups of particles show an irregular geometry, to describe these particles we decided to consider two characteristic dimensions of the aggregates: diameter and length. Then, we calculated the diameter of the spherical particles having

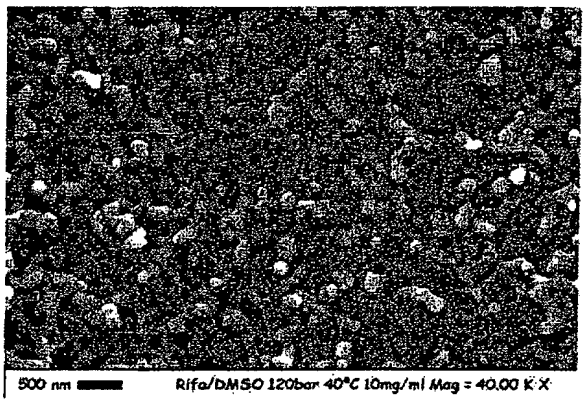

Fig. 4. SEM image of the micronized Rifampicin precipitated from DMSO at 120 bar, 40 °C and 10 mg/ml.

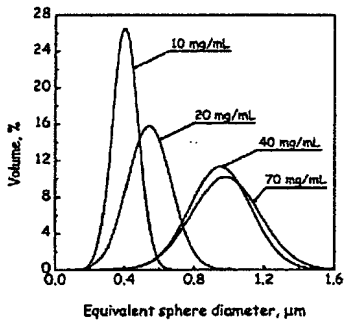

Fig. 5. PSDs of Rifampicin powders precipitated from DMSO. Calculations in terms of particle volume percentages.

the same volume as the correspondent aggregates. This data was used to evaluate the PSD of the powders in terms of volume percentage, the most representative distribution from the pharmaceutical point of view, since it is directly correlated to the drug dosage. PSDs obtained at different concentrations of Rifampicin in DMSO are reported in Fig. 5 and show that by increasing the solute concentration the mean PS increases and the PSD enlarges. The distributions are symmetric (Gaussian) and their mean ranges from 0.4 to 1 μm. Rifampicin particles obtained operating at 10 mg/ml DMSO are of particular interest since the PSD is very sharp and practically all particles fall within the range of injectable suspension drugs.

The second set of experiments was performed fixing the concentration of the liquid solution at 10 mg/ml and varying the precipitation pressure from 90 to 180 bar. For pressures higher than 120 bar, we obtained results similar to the ones obtained at 120 bar; whereas, in the tests performed at 90, 100 and 110 bar, we observed that, surprisingly, a different morphology of Rifampicin particles was produced: neat, spherical, micronic particles were obtained. An example of this morphology obtained operating at 100 bar is reported in Fig. 6. This morphology has been observed in some other cases of SAS precipitation (Reverchon et al., 1998, 2000b); but, this is the first time that it has been observed in connection with the one observed at pressures equal or larger than 120 bar; i.e. this change in morphology with precipitation pressure was not expected. Therefore, we decided to perform a new set of experiments at a pressure of 90 bar, varying the liquid solution concentration from 10 to 70 mg/ml to evaluate again the influence of concentration of Rifampicin on the morphology and PS. The results are exemplified in Fig. 7a and b, that allow a qualitative evaluation of the particle dimensions. The morphology does not change with concentration but, as for the experiments performed at 120 bar, the mean PS increases and the PSD enlarges. The quantitative PS analysis performed on SEM images showed that the mean particle diameter ranges in this case from 2.5 to 5 μm when evaluated on the volume basis. The PSDs are well represented by log-normal curves (Fig. 8) when calculated on the basis of the particle number percentages, and by a GCAS distribution (Fig. 9) when calculated on the basis of particle volume percentages. GCAS is an asymmetric curve commonly used in chromatography. The mode (the most frequent PS) of number of particles based distribution (Fig. 8) is always around 1 μm and varies only slightly with concentration, whereas the span of the distribution increases with this parameter. Volume based distributions (Fig. 9) move towards larger diameters with the increase of Rifampicin concentration in DMSO since the largest particles in the distribution assume a major relevance when computed in terms of volume.

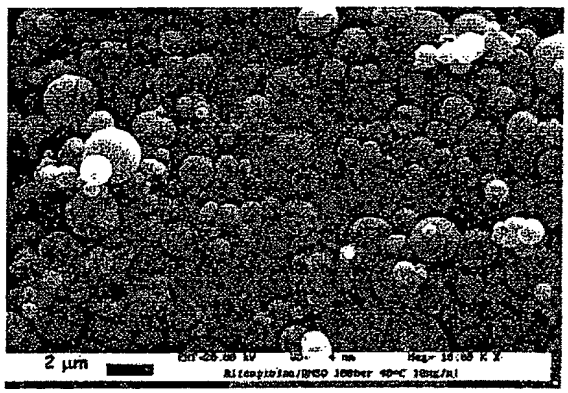

Fig. 6. SEM image of Rifampicin micronized from DMSO at 100 bar, 40 °C and 10 mg/ml.

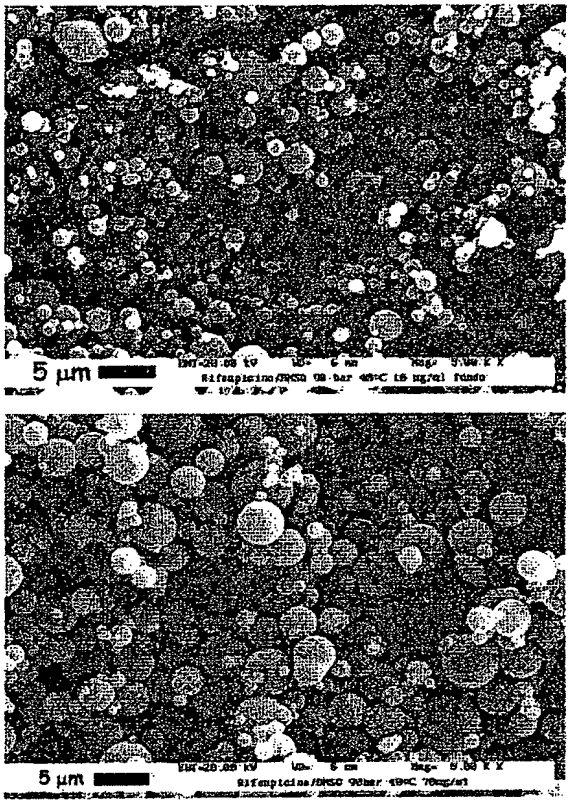

Fig. 7. SEM images taken at the same enlargement of micronized Rifampicin at 90 bar, 40 °C; (a) 10 mg/ml DMSO; (b) 70 mg/ml DMSO.

On the basis of results reported in Fig. 9, Rifampicin particles produced at concentrations of 10 and 20 mg/ml in DMSO could be good candidates for aerosol delivery since the PSD is narrow and under 5 μm in diameter; moreover, particles with diameters lower than 1 μm give a very small contribution to the overall PSD.

Occasionally, during SEM observations, we observed that some spherical particles randomly distributed in the sample were partly destroyed (Fig. 10). In these cases it was possible to observe the inner of these particles. The conclusion is that Rifampicin particles are not continuous, but formed by nanopieces strictly connected all together. This indication could be useful in the

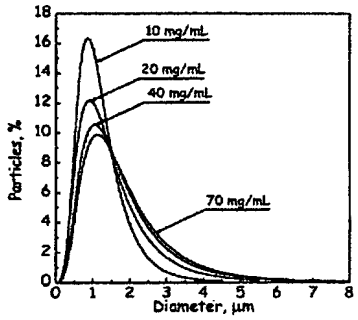

Fig. 8. PSDs of Rifampicin powders precipitated from DMSO at 90 bar, 40 °C; calculations in terms of particle number percentages.

understanding of the mechanism of particles growth during the SAS precipitation: micronic particles precipitated by SAS are tight aggregates of nanoparticles instead of the result of a single growth process.

The comparison of Rifampicin XRD patterns showed that Rifampicin was crystalline before processing and amorphous after SAS (Fig. 11). This difference can be explained by the very fast precipitation that can characterize the SAS that does not allow the organization of the compound in a crystalline form.

We also performed HPLC analysis on untreated and SAS processed Rifampicin: no modification occurred in HPLC retention peaks; therefore, the SAS process has not induced degradation of the drug (Fig. 12).

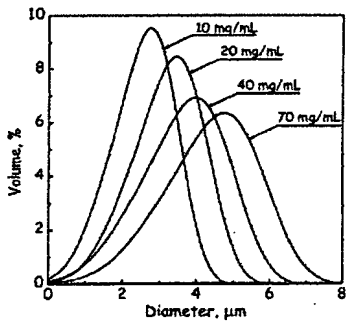

Fig. 9. PSDs of Rifampicin powders precipitated from DMSO at 90 bar, 40 °C; calculations in terms of particle volume percentages.

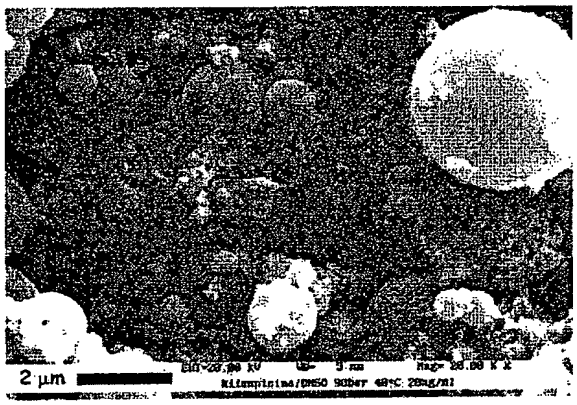

Fig. 10. SEM image of Rifampicin micronized from DMSO at 90 bar, 40 °C, 20 mg/ml.

In the attempt to clarify how different Rifampicin morphologies have been obtained operating SAS below or above 120 bar, we decided to repeat a selected series of experiments on the quartz windowed precipitator.

First, some experiments were performed to confirm information on the binary DMSO–CO_2 system behavior. We used the windowed precipitator and when we operated in the two phase conditions; i.e. at conditions in which the binary system liquid–supercritical CO_2 is not completely miscible, we observed a neat separation between the liquid and supercritical phase represented by a meniscus and the formation of a liquid jet that entered deeply in the fluid phase before its disappearance. When we repeated the same experiment in the single-phase region; i.e. at pressure and

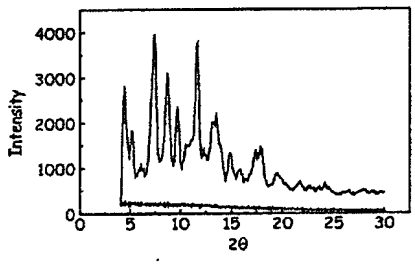

Fig. 11. Comparison of Rifampicin XRD patterns before and after SAS process. Upper trace: unprocessed. Lower trace: SAS processed.

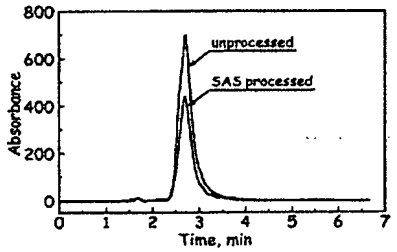

Fig. 12. Comparison of HPLC traces of untreated and SAS precipitated Rifampicin.

temperature conditions for which the binary system exhibits complete miscibility, the liquid jet was visible only in the immediate proximity of the injector. A further increase of pressure produced its almost complete disappearance. These results demonstrate that in the single-phase region the dissolution of the liquid into the supercritical phase is extremely fast and that the higher is the pressure the faster is the kinetics of liquid dissolution in the SCF. Therefore, the limiting step of the precipitation could not be the mass transfer but the thermodynamics; i.e. VLE at high pressure.

At 40 °C single phase behavior of the binary system starts at pressures slightly higher than 90 bar as previously observed on the basis of liquid expansion curves (Reverchon et al., 1998). Whereas, during the tests performed using the ternary system Rifampicin–DMSO–CO_2 and using the windowed vessel, we observed at 90 bar, 40 °C the formation of two phases almost as soon as we started the injection of the liquid solution. Although the injection of the liquid solution was continuous, this situation was stable; i.e. the volume occupied by the two phases was constant. The steadiness of the volumes allows us to suppose that the system was very close to thermodynamic equilibrium. Thus, despite the binary system DMSO–CO_2 is completely miscible at these conditions, the presence of Rifampicin induces the formation of a fluid and a liquid phase. The upper phase was transparent, while the lower one was red (the typical color of Rifampicin). The two phases were separated by a sharp and defined interface, Rifampicin precipitated from the lower phase starting from the separation meniscus.

Thus, two phases are formed, due to the presence of solute that modifies the high pressure VLE moving the mixture critical point (MCP) (i.e. the pressure at which the mixture is supercritical) towards higher pressures. It is possible to visualize this situation using Fig. 13. In this figure we report a semi-quantitative solubility diagram for the system DMSO–CO_2 (represented with a solid line; data adapted from Reverchon et al., 1998) and its hypothesized modification (represented with a dashed line) for the ternary system with the shift of the MCP to higher pressures, as our visual observations suggested when Rifampicin was added. However, Fig. 13 is only a simplification of the real behavior of the ternary system that should correctly be represented on a triangular diagram. Point A in Fig. 13 is located at conditions where we observed the two phases. Indeed, the mixture A splits in two parts in equilibrium, whose composition is given by two points on the boundaries of miscibility curve: a supercritical phase containing part of the liquid solvent and negligible quantities of Rifampicin, and a liquid one, containing part of CO_2 and almost all the solute. When Rifampicin precipitates from this expanded liquid phase, the morphology of spherical non-aggregated particles is produced. Increasing the pressure (departing from 90 bar), we observed that the higher was the pressure, the smaller was the level occupied by the lower phase. When the operating pressure was 120 bar, the lower phase vanished and the upper phase filled the whole volume of the chamber. At these conditions, Rifampicin precipitated from this unique fluid phase. Thus, when we operated at pressures larger than about 120 bar, we worked on point B in Fig. 13; i.e. above the MCP of the ternary system; we were at supercritical conditions with respect to the whole system and nanometric connected particles were produced (Fig. 4). Thus, the parameter controlling the observed change in SAS precipitates morphology is of thermodynamic nature and is connected to the high pressure phase equilibria characteristic of the Rifampicin–CO_2–DMSO system.

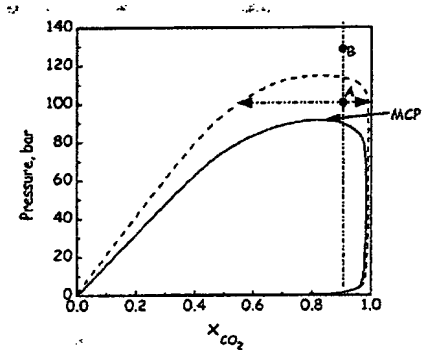

Fig. 13. High pressure solubility diagram at 40 °C for the binary system DMSO–CO_2 (solid line) and its hypothesized modification due to the presence of Rifampicin (dashed line). MCP is the mixture critical point, A is an operating point in the two phases region and B is an operating point into the one phase region.

4. Conclusions

Using SAS we produced micronic and nanometric particles of Rifampicin by varying the pressure of the process. Moreover, we can control the PS and the PSD of the particles by varying the concentration of the liquid solution.

At low pressures and concentrations (for example, at 10 mg/ml) only the 4.5% of the particles on volume basis (see also Fig. 9) has a diameter lower than 1 μm and no particles have a diameter larger than 5 μm; i.e. 95.5% of the volume of micronized particles ranges between 1 and 5 μm, that is the range suitable for aerosol delivery.

It is also possible to produce nanometric Rifampicin particles working at high pressure and low concentration of the liquid solution. For example, at 120 bar and 10 mg/ml (see also Fig. 5), only 12% of the particles has an equivalent diameter larger than 0.5 μm (on the volume basis). Therefore, it could be possible to consider these particles for the production of injectable suspensions.

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